

LAB QUALITY INTERNATIONAL **Eurachem**
A focus for analytical chemistry in Europe

Method validation in analytical laboratories

Presentation at
 A two-day training course
 ACCREDITATION OF ANALYTICAL, MICROBIOLOGICAL AND MEDICAL
 LABORATORIES - ISO/IEC 17025:2017 AND ISO 15189:2012

20 - 21 February 2020 in Nicosia, Cyprus

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Securing a method is fit for the purpose

Need to find out about some characteristics of a material (sample) ...and **make a decision**

Customer / client

METHOD SOLUTION!

Laboratory

Competent in analytical chemistry ... must **make a decision** on best method for the task

Develop (modify) **Validation!**

Verification! Analytical R&D Stand. Methods

Reliable basis for making decisions! ..if the method is valid!!!

Method Validation

✓ the process of proving that an analytical method is acceptable for its intended purpose

An analytical method is (mostly) a process!

- Process
 - from start material to final result
- ✓ Few or many steps
- ✓ Physical / chemical principles
- ✓ Mostly comparative!!!
- ✓ Many influence parameters
- ...and finally:
 - ✓ Result often depending on method
 - but will always be depending on the sample and its character
 - whether it tells something about the original material

Analytical methods and reliability


- ✓ Analytical results are used as basis for decisions
 - - is this raw material in accordance with specifications?
 - - are the limits for pollution exceeded?
 - - does this food contain the promised amount of meat ?
 - - has this patient got hepatitis?
 - - etc. etc. etc.
- ✓ Reliable results (appropriate for the situation) are crucial for coming to the right decision
- ✓ The analytical method is of decisive importance
 - Selection of appropriate method
 - Choice of an appropriate level of quality (~ MU on result)
- ✓ The principle of **"Fitness for Purpose"** !
 - ⇔ **Method Validation !**

Method validation ⇔ Fitness for Purpose

- ✓ The Method Validation must secure that the method is fit..
 - **For solving the analytical task for the client**
 - ... but also...
 - **For being applied in the laboratory (routinely!?)**
- ✓ Nice to have / Need to have - be realistic!!
- ✓ Requirements / Expectations
 - What is taken for granted?!
- ✓ Information needed as basis for making important decisions
 - BOTH for the client AND the laboratory!!
 - Must be trustworthy!
 - Blind faith – or documented evidence ?

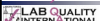
Still a lot of new challenges in the field

- ✓ Advanced/New techniques
 - Multi-parameter methods
 - Multi-matrix methods
 - Verification of test kits/automated analysis (black box)
 - Non-targeted methods
- ✓ Setting performance requirements
- ✓ How to do a proper validation study – securing “Fitness for Purpose” both outside AND inside the lab.?
 - Planning including efficient design of experiments
 - Elaboration of the validation protocol
 - How to plan verification based on info in the standard
- ✓ Method validation/instrument qualification
- ✓ **Requirements for accreditation of laboratories!** ⇨




Requirements in ISO/IEC 17025:2017

- ✓ *The laboratory shall **verify** that it can **properly perform methods** before introducing them by ensuring that it can **achieve the required performance**.*
 - **Records of the verification** shall be retained.
 - *If the method is revised by the issuing body, verification shall be **repeated to the extent necessary**.*
- ✓ ISO/IEC 17025 does not give any requirements on how the verification should be done – but gives a definition (3.8):
 - **Provision of objective evidence** that a given item fulfils **specified requirements**
 - A very generic definition – but gives a couple of examples
 - 1) *Confirmation that performance properties or legal requirements of a measuring system are achieved.*
 - 2) *Confirmation that a target measurement uncertainty can be met.*




Requirements in ISO/IEC 17025:2017

- ✓ Definition of validation according to the standard (3.9):
 - **Verification**, where the **specified requirements are adequate for an intended use**
- ✓ And the requirements are:
 - *The laboratory shall validate **non-standard methods, laboratory-developed methods and standard methods used outside their intended scope or otherwise modified**.*
 - *The validation shall be **as extensive as is necessary** to meet the needs of the given application or field of application.*
- ✓ The standard is suggesting some possible techniques for **validation through comparison** and **in-house validation**
- ✓ Procedure, requirements, evaluation and conclusion of a validation study must be **documented**



The Eurachem Guide

Widely applied guidance on method validation:



The Fitness for Purpose of Analytical Methods
 A Laboratory Guide to Method Validation and Related Topics

- ❑ Issued 1st version of the "Fitness For Purpose Guide" in 1998
- ❑ 2nd revised edition, October 2014!
- ❑ WG have issued two supplementary documents
 - **Planning and Reporting Method Validation Studies**
 - **Blanks in Method Validation**
- ❑ 3rd edition under preparation

All documents can be downloaded for free from www.eurachem.org

Performance characteristics

Table 2 – Overview of performance characteristics commonly evaluated during method validation

✓ The parameters, which are normally object for investigation(s) as part of a method validation study!

Performance characteristic
Selectivity
Limit of detection (LOD) and limit of quantification (LOQ)
Working range
Analytical sensitivity
Trueness <ul style="list-style-type: none"> • bias, recovery
Precision <ul style="list-style-type: none"> • repeatability, intermediate precision and reproducibility
Measurement uncertainty ^a
Ruggedness (robustness)

^a Strictly, measurement uncertainty is not a performance characteristic of a particular measurement procedure but a property of the results obtained using that measurement procedure.


Phases in the work of validation

- ✓ (Analytical task laid down)
- ✓ (Method chosen)
- ✓ Set up the requirements for the method
- ✓ Background for validation in the laboratory (checklist!)
- ✓ Decide on the extent of the validation (maybe step by step)
- ✓ Write the validation protocol(s)
- ✓ Carry out the experimental work in the laboratory
- ✓ Calculate and evaluate results
- ✓ Prepare a report on the validity of the method.
- ✓ **According to the laboratory's procedure for method validation !!!**

Procedure for method validation (example)



1. Purpose and scope
2. Responsibilities and users (qualifications!)
3. Procedure
 1. Choice of method
 2. List background for validation of method
 3. Extent of validation
4. **Validation protocol(s)**
5. Experimental work in the lab.
6. Treatment of results
7. Reporting

4. Documentation of method
5. Records




Protocol for method validation

- ✓ Validation protocols deals with the single method validation
- ✓ Exact description of the "road map" for the experimental work
 - Covering the relevant method characteristics
 - Necessary extent of work
- ✓ In case of need for any changes and/or additional experiments, it must be put in an amendment to the original protocol.



Planning of the experiments

- ✓ Experiments for
 - Measuring range
 - Linearity
 - Trueness
 - Precision
- ✓ ... can be combined in an overall plan using the results from the single experiments for multiple purposes.
- ✓ Experiments for robustness testing can with advantage be based on some statistical principles for experimental design
 - E.g. the Placket-Burman design!




Elaboration of a validation protocol

- ✓ Individual - systematically (if convenient)
- ✓ Laboratory's paradigm should be stated in procedure
- ✓ Results of decision regarding extent of validation
- ✓ Cover all relevant validation parameters
 - Which samples (materials) to measure
 - Which levels of concentration
 - How many (regarding a min. number of DoF)
 - Time schedule (NOTE: laboratory reproducibility)
- ✓ Combine experiments if possible
- ✓ Overall time schedule for the whole validation process

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Protocol for method validation

- ✓ Validation protocols deal with the single method validation
- ✓ Exact description of the "road map" for the experimental work
 - Covering the relevant method characteristics
 - Necessary extent of work
- ✓ E.g. by following the new Eurachem supplement to the Fitness for Purpose Guide:


Planning and Reporting Method Validation Studies
Supplement to Eurachem Guide on the Fitness for Purpose of Analytical Methods

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Points to consider when planning a validation study


- ✓ The method to be validated
 - A detailed written procedure
 - Method development completed
- ✓ Critical steps in the method and instrument requirements
 - Study the analytical process (flow diagram)
 - Identify any critical steps, like e.g.
 - specific requirements for equipment
 - sensitivity to influence factors (environment, settings etc.)
 - special qualifications/experience needed for handling
- ✓ Supporting information
 - Previous experience with performance of (similar) method
 - Data from IQC or participation in ILCs / PTs

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Points to consider when planning a validation study (cont'd)

- ✓ The method to be validated
 - A detailed written procedure
 - Method development completed
- ✓ Critical steps in the method and instrument requirements
 - Study the analytical process (flow diagram)
 - Identify any critical steps, like e.g.
 - specific requirements for equipment
 - sensitivity to influence factors (environment, settings etc.)
 - special qualifications/experience needed for handling
- ✓ Supporting information
 - Previous experience with performance of (similar) method
 - Data from IQC or participation in ILCs / PTs


(cont.)



Background for method validation (list)


- ✓ Tools available for the validation
 - Reference materials (relevant matrix - e.g. CRMS)
 - Real samples (stable)
 - Spiked materials (feasible?)
 - Blanks (See supplement "Blanks in Method Validation")
 - "Reference" method(s) for comparison
 - ILC / PT (in the period for the validation study)
- ✓ Planning of study / Experimental design
 - Where to start (Selectivity first – if relevant)
 - Combined studies over planned period
- ✓ Miscellaneous
 - Any spec. conditions relevant for the performance/extent of validation

Checklist !



Treatment of results from validation

- ✓ Use of statistical tools for.....
- ✓ Evaluation – and possibly exclusion – of outliers
- ✓ Calculation of averages, standard deviations and number of DoF
 - If possible: pooling of standard deviations from different days
- ✓ Regression analysis on calibration - and linearity-data
 - Weighted (if relevant)
 - Charting – and visual inspection / Coefficient of correlation
 - Non-linear calibration function?
- ✓ Precision data expressed as st.dev., %RSD or as 95% confidence interval
- ✓ ANOVA (ANalysis Of Variance) for distinguishing contributions to intermediate precision
- ✓ Maybe t-/ F-testing for evaluation in relation to requirements



Extent of validation studies

- ✓ It has to be decided by the laboratory which performance characteristics need to be investigated as part of a validation study
 - AND how detailed the investigation of a single performance characteristic should be
- ✓ Should "in principle" be specified by the customer/user!
- ✓ A careful consideration of the analytical specification given in the scope of the documented procedure provides a good base on which to plan the validation process.
(may sometimes also be sector-specific; E.g. pharm. Sector)
- ✓ ISO/IEC 17025:2005 (clause 5.4.5.3):
 - *Validation is always a balance between costs, risks and technical possibilities.*

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Validation / Verification
(Acc. to ISO 9000)

- ✓ **3.8.5 validation**
 - confirmation, through the provision of **objective evidence** (3.8.1), that the **requirements** (3.1.2) for a specific intended use or application have been fulfilled
- ✓ **3.8.4 verification**
 - confirmation, through the provision of **objective evidence**, that specified **requirements** have been fulfilled
- ✓ **3.8.1 objective evidence**
 - data supporting the existence or verity of something
 - NOTE: Objective evidence may be obtained through observation, measurement, **test**, or other means

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Validation vs. Verification !

Within the Eurachem Guide the following distinction is made clear and valid throughout the guide:

- "A laboratory may **adopt a validated procedure** which, e.g. has been published as a standard, or buy a complete measuring system to be used for a specific application from a commercial manufacturer."
- "In both these cases, **basic validation work has already been carried out** but the laboratory will still need to confirm its ability to apply the method."
- "This is **verification**."
- I.e. "...some experimental work must be done to demonstrate that the method works in the end-user's laboratory"
- But "...the workload is likely to be considerably less"

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Verification / implementation of standard met.

- ✓ Procedures for how to verify that the laboratory can perform a standard method according to specification needs to be established.
 - Internally
 - Repeated testings over 2-3 weeks (changing conditions)
 - Use of reference material – IF possible
 - Getting familiar with the performance of the method (depending on how complicated the method is) &
 - Externally
 - Comparisons with other laboratories (internally/externally)
 - Participate in a PT – IF possible
 - Document
- ✓ Elaborate the necessary supplementary SOPs to secure good – and consistent – application of the method

Currently ensuring Validity of test results

- ✓ **Follow-up on validation/verification** by Quality Assurance of results
- ✓ According to the ISO/IEC 17025:
 - *The laboratory shall have a procedure for **monitoring the validity of results.***
 - *The resulting data shall be recorded in such a way that **trends are detectable** and, where practicable, **statistical techniques** shall be applied to review the results.*
- ✓ Internally
 - Establish IQC (routinely testing of control materials and plotting results into control charts).
 - Warning and control limits / Decision rules
- ✓ Externally
 - Selecting - and planning participation in – PT schemes (as possible/relevant)

Checklist for a validation study

(Appendix 1 to supplement document, “*Planning and Reporting of Validation studies*”)

A. Analytical requirement			
A.1	Analyte specified?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
A.2	Measurand specified?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
A.3	Matrix and form of samples specified?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
A.4	Expected levels/required working range specified?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
A.5	Purpose of method well understood?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
A.6	Use of results clearly specified?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
A.7	Any specific regulatory requirements?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
A.8	Are results to be used for critical decisions?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
A.9	Performance characteristics to be studied identified?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
A.10	Target values for performance characteristics stated?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
A.11	Extent of routine use of the method known?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
A.12	Any deadline for start of routine use of method?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
Other comments/actions:			
B. Purpose of validation study			
B.1	Purpose of validation exercise stated?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
B.2	Method to be validated for use in another laboratory?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
Other comments/actions:			
C. Knowledge of selected method			
C.1	Method/similar methods well known in lab?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
C.2	Clear and unambiguous method description available (e.g. standard operating procedure)?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
C.3	Any known/foreseen critical steps?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
C.4	Any supplemental standard operating procedures required?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
C.5	Any health/safety issues?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
Other comments/actions:			

D. Specific requirements for performing the method			
D.1	Any specific requirements for sample handling/storage?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
D.2	Any specific requirements for sample preparation?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
D.3	Any specific requirements for equipment calibration?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
D.4	Any specific requirements for environmental monitoring?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
Other comments/actions:			
E. Competence for validation			
E.1	Responsible person for the study appointed?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
E.2	Analyst(s) carrying out validation familiar with the method?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
E.3	Supplementary training required?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
E.4	Supervision during validation required?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
Other comments/actions:			
F. Equipment and facilities			
F.1	Particular equipment required for sample preparation?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
F.2	Required measuring equipment available?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
F.3	Measuring equipment properly calibrated?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
F.4	Measuring equipment properly maintained?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
F.5	Facilities appropriate for the application of the method?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
F.6	Environmental conditions under control?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
Other comments/actions:			
G. Tools available for validation			
G.1	Suitable blanks available?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
G.2	RMs/CRMs available?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
G.3	Spiking of samples possible/required?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
G.4	Surplus test samples available?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
G.5	Stability of validation materials under control?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
G.6	Reference method(s) available?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
Other comments/actions:			

H. Evaluation of individual performance characteristics			
H.1	Performance target specified?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
H.2	Materials to be analysed specified and sufficient material available?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
H.3	Experimental plan defined (number of replicates, order of analysis)?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
H.4	Data analysis defined (including statistical tests)?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
H.5	Criteria for assessing fitness for purpose specified?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
Other comments/actions:			
I. Supplementary information to support assessment of method performance			
I.1	Any historical data available (e.g. IQC or results from routine application of method)?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
I.2	Possible to participate in PT during validation?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
I.3	Possible to participate in/arrange other ILC?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
Other comments/actions:			
J. Approval of validation plan			
J.1	Validation plan signed off by appropriate person?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
Other comments/actions:			
K. On completion of study			
K.1	Assessment of fitness for purpose completed for each performance characteristic and method as a whole?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
K.2	Validation report signed off?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
K.3	Final method documentation (e.g. standard operating procedure) prepared and signed off?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
K.4	Ongoing quality control requirements established?	<input type="checkbox"/> YES <input type="checkbox"/> NO	Action:
Other comments/actions:			